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Effects of Exposure to Peroxyacetyl Nitrate on Susceptibility to Acute and Chronic Bacterial Infection

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16. ABSTRACT

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JOURNAL ARTICLE

EFFECTS OF EXPOSURE TO PEROXYACETYL NITRATE ON SUSCEPTIBILITY TO ACUTE AND CHRONIC BACTERIAL INFECTION

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A significant increase in mortality due to acute respiratory pneumonia caused by inhalation of Streptococcus pyogenes *aerosol was seen after a single 3-h exposure of mice to 14.8–28.4 mg/m³ peroxyacetyl nitrate (PAN). The excess mortality ranged from 8 to 39% and the decrease in survival time from 2.4 to 7.9 d. A single exposure to 25.0 mg/m³ PAN resulted in a significant increase in total number of cells lavaged from the lungs but somewhat decreased levels of adenosine triphosphate (ATP) in alveolar macrophages. Exposure to 7.4 mg/m³ PAN for 3 h/d, 5 d/wk, for 2 wk resulted in a reduced total count of free pulmonary cells and a significant reduction of ATP levels in alveolar macrophages but had no effect on mortality or survival rate. Scanning electron microscopic observations of the respiratory tract after both single and multiple exposures to PAN showed raised and sloughing nonciliated cells in the nasal cavities and tracheas and presence of excess mucus. Six daily 3-h exposures to 25.0 mg/m³ PAN did not produce any marked changes in a chronic respiratory infection in mice as measured by* Mycobacterium tuberculosis *lung titers.*

INTRODUCTION

Peroxyacetyl nitrate (PAN) is the first member of a series of peroxyacyl nitrates ($R\text{-}\overset{O}{\overset{\|}{C}}\text{-}OONO_2$) that are important constituents of photochemical air pollution. PAN is a highly oxidized, unstable organic N compound that is present in the atmosphere as the result of ultraviolet irradiation of certain unsaturated hydrocarbons in the presence of oxides of N. It was the first

The authors wish to thank Mrs. Julie Deisinger for her excellent technical assistance. Dr. Max Lustig established the system for the production and generation of PAN. Mr. William Sumida assisted in the production of PAN. Ms. Kathy Ketels performed the scanning electron microscopic examinations and Ms. Catherine Aranyi performed the analyses on free cells lavaged from the lungs.

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pure component of photochemical smog recognized as causing plant damage and eye irritation (Stephens et al., 1961).

Campbell et al. (1967) reported at the LC^{50} for mice for a 2-h exposure was 524–742 mg/m^3 PAN. The LC^{50} was closely related to the age of the mice and the environmental temperature maintained during the exposure. A higher susceptibility to PAN was seen at 32°C than at 27°C, and 4- to 6-mo-old mice were more susceptible than 2- to 3-mo-old ones. Campbell et al. (1970) reported a depression of voluntary motor activity in mice during exposure to ~ 39.6 mg/m^3 PAN. Dungworth et al. (1969) reported that daily 6-h exposures of mice to 74.2 mg/m^3 PAN for 6 mo caused 18% mortality, weight loss, and damage to the respiratory tract tissue. Other studies indicate that the toxicity of PAN for mice is greater than that of SO_2, similar to that of NO_2, and lower than that of O_3 (National Air Pollution Control Administration, 1970).

The purpose of this study was to determine the effects of PAN on susceptibility to acute and chronic respiratory infections. An acute infection was established in mice by respiratory challenge with *Streptococcus pyogenes* aerosols. A chronic infection was initiated by respiratory challenge with an aerosol of *Mycobacterium tuberculosis* attenuated human strain RIRv. This *M. tuberculosis* strain is the most virulent of the attenuated human strains (Lefford et al., 1973) and has been shown to grow in the liver and spleen of rats.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley CF_1 albino mice, C57BL/6 black mice, and CD_2F_1 hybrid brown mice (Murphy Breeding Laboratory, Plainfield, Ind.), 6–16 wk old, were used. The mice were housed in groups of 10 in stainless steel shoebox cages and provided food and water *ad libitum*.

Infectious Challenge

Mycobacterium tuberculosis RIRv (Trudeau Institute no. 205, Saranac Lake, N.Y.) was grown in Proskauer and Beck broth at 37°C until confluent pellicle growth was obtained. A loopful of the pellicle was transferred to 50 ml Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) containing 0.1% Tween 80 and incubated for 7–10 d until the culture was turbid. The unwashed culture was homogenized with a Teflon pestle and 50-ml homogenizing vessel. The titer was determined by serial dilution in 0.1% phosphate-gelatin [Bacto hemagglutination buffer (Difco) with 0.1% gelatin], plating 0.1-ml portions onto Dubos oleic acid albumin (OAA) agar containing 50 u/ml penicillin G and 0.1% cyclohexamide, and counting the colonies after 3 wk incubation at 37°C. The homogenized cultures were frozen in 8-ml portions at -70°C.

For aerosol dissemination, 6 ml thawed culture was diluted approximately 1:10 in Middlebrook 7H9 broth containing 1.5% antifoam A (Dow Chemical Co., Midland, Mich.). The bacterial suspension was disseminated at a rate of 0.75 ml/min from a DeVilbiss (model 841) continuous-flow nebulizer, using

a primary filtered air flow of 8 l/min. The aerosol was disseminated into a 30 × 30 × 90 cm Plexiglas chamber installed with a microbiological safety hood.

For the infectious challenge, groups of 48–60 mice were housed in individual compartments of specially designed Al wire cages and were exposed for 20 min to the aerosol. The inhaled dose was determined by homogenizing lungs from three mice immediately after the challenge in 2 ml 2% albumin and plating 0.2 ml of 10-fold phosphate-gelatin dilutions onto OAA agar. The plates were incubated for 4 wk at 37°C. The number of bacteria in a total volume of 2.2 ml was considered the number of viable *M. tuberculosis* per animal. Similarly, groups of six guinea pigs were exposed to this infectious aerosol for 2 min. This length of exposure was based on the breathing rate of guinea pigs compared to mice and on the experience of another investigator who had obtained low inhaled doses (Wiegeshaus et al., 1970).

Streptococcus pyogenes group C (Lancefield strain) was passed in mice, isolated from hearts, incubated for 18 h at 37°C in Todd Hewitt broth, and frozen in 1-ml portions at -70°C. Methods of exposure of mice to the aerosol of *S. pyogenes* have been described (Ehrlich et al., 1977).

Generation and Monitoring of Peroxyacetyl Nitrate

PAN was synthesized as described by Kacmarek et al. (1978). Pure PAN was introduced into a 60 × 70 × 40 cm Plexiglas exposure chamber by passing N_2 at a rate of 300–700 ml/min over liquid PAN held in a modified cold trap at -30°C in Freon 12. An in-line filter and hydropurge removed moisture and oil from the N_2 line. The chamber temperature was maintained at 26.5 ± 1.0°C. Concentrations of PAN in the chamber were maintained by adjusting flow rates of N_2 and air and were expressed in milligrams per cubic meter (ppm × 4.95 = mg/m^3). The relative concentration of PAN in the chamber was monitored continuously with a Mast analyzer containing KI solution, with an attached stripchart recorder (model 725-3CS, Mast Development Co., Davenport, Iowa). An NO_X Chemiluminescent Analyzer (model 7101-B, Bendix Corp., Ronceverte, W. Va.) was used to calibrate the Mast analyzer.

The concentration and purity of PAN in the chamber were also analyzed in an infrared (IR) spectrophotometer (Perkin-Elmer, model 283) equipped with a wave marker and a repetitive scan device. To determine the PAN concentration in the animal exposure chamber, a slight positive pressure was maintained in the chamber and air was exhausted through the IR cell. After a large volume of air (compared to the cell volume) passed through the cell, the cell was closed off with a valve and placed in the spectrophotometer. The PAN concentrations determined by IR analysis were in the range of concentrations of NO_X determined by the Mast analyzer-NO_X Chemiluminescent Analyzer measurements.

Lung Lavage

Free pulmonary cells were obtained by tracheobronchial lavage from excised lungs of mice killed within 1 h after the last exposure to the pol-

lutant (Fenters et al., 1979). Total and differential counts and viability of cells isolated from the lavage fluids by centrifugation were examined (Coffin et al., 1968; Weissbecker et al., 1969). The adenosine triphosphate (ATP) concentration in the lavaged cells was determined by bioluminescence (Waters et al., 1975b), using a Dupont 760 luminescence biometer. *In vitro* phagocytosis of polystyrene latex particles by alveolar macrophages (AMs) was evaluated by light microscopy (Gardner et al., 1973). The AMs were mixed with the latex particles in a 1:100 ratio. Surface morphology and attachment to glass substrate of the AMs were examined by scanning electron microscopy (Waters et al., 1975a) in a Kent-Cambridge Mark II scanning electron microscope.

Respiratory Tract Morphology

Nasal cavities, tracheas, and lung tissue were prepared for scanning electron microscopic examination as described by Ketels et al. (1977). Samples of the same tissues were cut at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy.

Chronic Infection Parameters

Mycobacterium tuberculosis titers in the lungs were determined weekly for 8 wk in groups of five mice killed at each interval. Dilutions of homogenized tissue suspensions were made in 0.1% phosphate-gelatin and 0.2-ml portions from two dilutions were plated in triplicate onto OAA agar. Plates were incubated in CO_2-impermeable 29 × 46 cm Supervac bags (VacPac Manufacturing Co., Baltimore, Md.) at 37°C. Plates containing 1–350 colonies were counted after 3 and 4 wk incubation. Colony forming units (CFU) from all plates were averaged and multiplied by the dilution factor to give the total *M. tuberculosis* in 2.2 ml homogenized tissue suspension. The $\log_{10}$ of the CFU from each lung was averaged for each time period and the concentrations of *M. tuberculosis* in the experimental groups compared with those in the controls by Student's *t*-test.

Acute Infection Parameters

Mice challenged with *S. pyogenes* were observed over a 28-d period after the infectious challenge. The percent mortality and relative mean survival rate (RMSR) were determined as described by Fenters et al. (1979). The significance of the observed differences in mortality rates was determined by the chi-square test with a 2 × 2 contingency table.

The pulmonary clearance rate of inhaled viable *S. pyogenes* was determined at various time periods. Groups of four mice were sacrificed at 0.1, 2, 3, and 4 h after the challenge; the lungs aseptically removed, weighed, and homogenized in 2.0 ml peptone water; and the homogenates plated in 0.1-ml portions in duplicate onto blood agar plates. Colonies were counted after 48 h incubation at 37°C. The number of *S. pyogenes* per gram of lung (wet weight) was determined for each exposure condition. The initial deposition

(0 h) was designated at 100%. At each time interval the number of *S. pyogenes* per gram of lung was expressed as percent of the initial dose. The clearance rate $t50$—the time required for 50% of the inhaled viable bacteria to be cleared from the lungs—was determined on a semilogarithmic regression after converting percent recoveries to $\log_{10}$ values. The slopes of the regression lines for the experimental and control groups were compared by Student's t-test.

RESULTS

Effect on Resistance to Streptococcal Pneumonia

Mortality and Survival. Mortality rates and mean survival times of mice after a single 2- or 3-h exposure to various concentrations of PAN and challenge within 1 h with *S. pyogenes* aerosol are shown in Table 1. Significant increases in mortality and decreases in survival time were seen in mice exposed to PAN at 14.8-28.4 mg/m^3. The mean excess mortality rate ranged from 8 to 39%. The decrease in survival time ranged from 2.4 to 7.9 d. No difference in mortality was observed between different strains of mice or mice of different ages (6-16 wk). The mean mortality of mice exposed for 2 or 3 h to 0.98 mg/m^3 O_3 and challenged with the infectious aerosol, which were included in the experiments as positive controls, was 38%. Thus PAN was less effective than O_3 in reducing the resistance of mice to the streptococcal pneumonia.

To further assess the effects of short-term exposure to PAN, the concentration × time (CT) index, representing the products of PAN concentration and duration of exposure, was calculated. The percent changes in mortality observed at each exposure condition were subjected to regression analysis and the resulting least-squares lines plotted against the CT index. The relation between excess mortality due to streptococcal pneumonia and the CT index can be seen in Fig. 1 (correlation coefficient $r = 0.95, p \leq 0.01$).

Daily exposure for 3 h/d, 5 d/wk, to 4.9 mg/m^3 PAN for 2 and 3 wk (10 and 15 exposures) or to 7.4 mg/m^3 for 2 wk (10 exposures)—CT index = 147, 220.5, or 222—had no appreciable effect on mortality or survival time (Table 2). This absence of effect could be due to tolerance, as reported for exposures to low concentrations of O_3. Apparently the concentration of PAN is more important than the duration of exposure in producing toxic effects, since a CT index of ~ 44 caused significant increases in mortality on a single exposure at the higher concentrations. Gardner et al. (1979) similarly observed for NO_2 exposures that concentration was more important than duration in producing increased mortality from acute bacterial infection.

Lung Clearance of Viable Inhaled *S. pyogenes*. The rates of clearance (t^{50}) of inhaled viable *S. pyogenes* from lungs of mice after single and multiple 3-h exposures to PAN were not significantly different from those of mice exposed to air (Table 3). After single 3-h exposures to 14.8 or 19.8

TABLE 1. Mortality and Survival Rates of Mice[a] Exposed for 2 or 3 h to PAN and Challenged within 1 h with *Streptococcus* Aerosol

PAN Exposure			Mortality					RMSR (d)		
			Air		PAN					
Concentration (mg/m^3)	Time (h)	CT index[b]	D/T[c]	%	D/T	%	Change (%)	Air	PAN	Change
14.8	3	44.4	6/90	7	14/91	15	+ 8[d]	26.5	24.1	-2.4
19.8	3	59.8	4/147	3	23/142	16	+13[e]	27.4	24.6	-2.8[e]
22.3	2	44.6	6/49	12	13/50	26	+14[d]	26.0	22.5	-3.5[e]
24.7	3	74.1	40/150	27	84/150	56	+29[e]	22.3	14.7	-7.6[e]
27.2	2	54.4	9/100	9	23/100	23	+14[e]	27.2	23.0	-4.2[e]
28.4	3	85.2	30/201	15	105/196	54	+39[e]	23.2	15.3	-7.9[e]

[a] CD_2F_1 and CF_1 mice aged 6–16 wk.
[b] Concentration × time.
[c] Dead/total.
[d] Significant change from corresponding infected mice exposed to filtered air ($p < 0.1$).
[e] Significant change from corresponding infected mice exposed to filtered air ($p < 0.05$).

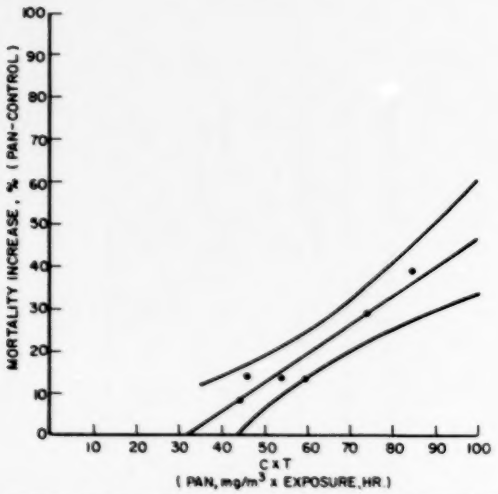

FIGURE 1. Relation between excess mortality due to streptococcal pneumonia in mice and CT index for PAN exposure (mean and 95% confidence limits).

mg/m³ PAN, clearance rates were similar to those in control mice exposed to air, although mortality rates were significantly higher in mice exposed to the same concentrations of PAN. On repeated daily exposures to PAN the clearance rates were also similar to those of mice exposed to air.

Effect on Resistance to Tuberculosis

Mycobacterial Titers in Lungs. Changes in resistance to the infection, as determined by *M. tuberculosis* titers in lungs, were studied in mice exposed to PAN and compared to those in mice exposed to filtered air. In all experiments the mice were first challenged with *M. tuberculosis* aerosol and then exposed to various PAN regimens.

In mice exposed to filtered air, the inhaled dose of *M. tuberculosis* ranged from 45 to 125 CFU per lung and there was no apparent increase in titer 1 wk after the infectious challenge. Thereafter, the mycobacteria titer showed a continuous increase, reaching a peak 4-5 wk after the challenge. In general, the titers remained approximately constant for an additional 2-3 wk and decreased slowly thereafter. This growth pattern is similar to that observed for the virulent strain of *M. tuberculosis*, H37Rv, in lungs of guinea pigs exposed by the aerosol route (Smith et al., 1970).

Daily 3-h exposure to 25.0 mg/m³ PAN for 6 d beginning 1 wk after the challenge with *M. tuberculosis* did not alter the pattern of bacterial growth in the lungs of mice. The *M. tuberculosis* titer in lungs reached a peak approximately 4 wk after the infectious challenge, and a slight decrease was seen during an additional 6 wk observation period.

TABLE 2. Mortality and Survival Rates of Mice Exposed 3 h/d, 5 d/wk, for 10 or 15 d to PAN and Challenged within 1 h with *Streptococcus* Aerosol

PAN exposure		Mortality					RMSR (d)		
		Air		PAN					
Concentration (mg/m^3)	Duration (d)	D/T	%	D/T	%	Change (%)	Air	PAN	Change
4.9	10	31/160	19	41/159	26	+7	23.8	22.6	-1.2
4.9	15	2/30	7	2/27	8	+1	26.6	26.4	-0.2
7.4	10	17/75	23	12/77	16	-7	23.2	25.0	+1.8

TABLE 3. Clearance of Inhaled *Streptococcus* from Lungs of Mice Exposed to PAN

PAN exposure		Mice		Clearance rate t_{50}[a]	
Concentration (mg/m³)	No. of 3-h exposures	Strain	Age (wk)	Air	PAN
14.8	1	CD_2F_1	9-12	1.13	1.10
19.8	1	CD_2F_1	5	0.66	0.84
4.9	10	CF_1	16	1.10	1.25
7.4	10	CF_1	6-9	0.96	1.09
4.9	15	CF_1	8	1.20	1.00

[a]Time in hours required to clear 50% of inhaled viable bacteria.

Effects on Free Pulmonary Cells

Limited experiments were conducted to determine the effects of exposure to PAN on free cells lavaged from lungs of mice 6-8 wk old. The two treatment groups included mice exposed for 3 h to 19.8 mg/m³ PAN and mice exposed daily, 3 h/d, 5 d/wk, for 2 wk to 7.4 mg/m³ PAN (Table 4).

The single 3-h exposure resulted in a significant ($p < 0.05$) increase in total cell counts, some decrease in ATP levels, but no effect on the differential counts or the viability or phagocytic activity of the AMs. The multiple exposures resulted in some decreases in total cell counts and a significant ($p < 0.05$) decrease in ATP; the differential counts and the viability and phagocytic activity of the AMs were not affected.

Cells lavaged from the lungs of mice in the various treatment groups were incubated to determine their ability to attach to a glass substrate. Scanning electron microscopy indicated that after a single 3-h exposure to 19.8 mg/m³ PAN a large number of AMs attached to the glass; no surface structural changes compared to cells obtained from mice exposed to filtered air were apparent. After daily 3-h exposures to 7.4 mg/m³ PAN, 5 d/wk, for 2 wk very few cells were found on the glass substrates and therefore morphological changes could not be determined. Thus it appears that the ability of AMs to attach to the glass substrate was impaired. Although no effects on macrophage mortality and survival rate were seen, the results of the multiple-exposure regimen indicate that the pulmonary defense system of the host was compromised.

Respiratory Tract Tissue Morphology

On scanning electron microscopic examination, an abnormal raised appearance of nonciliated epithelial cells of the nasal cavity was the major change seen in mice exposed for 3 h to 25 mg/m³ PAN (Fig. 2). The lungs and trachea appeared normal on both light and scanning electron microscopic examination. A similar raised appearance of nonciliated epithelial

TABLE 4. Effect of Exposure to PAN on Free Cells Lavaged from the Lungs of Mice[a]

Exposure		Cell count								Alveolar macrophage					
		Total × 10^6		Differential (%)						Viability (%)		Phagocytosis[b] (%)		ATP	
				Macrophage		Lymphocytes		Neutrophils							
Concentration (mg/m^3)	No. of 3-h exposures	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Se	Mean	SE
0	1	6.92	0.72	97.0	1.5	3.0	1.5	<1		95.2	1.3	91.0	2.0	1.03	0.05
19.8	1	9.95[c]	1.03	97.7	0.9	2.3	0.9	<1		96.7	0.5	88.0	1.0	0.89	0.05
0	10	8.86	0.86	97.7	0.7	2.3	0.7	<1		95.2	0.9	89.9	1.8	0.98	0.05
7.4	10	6.11	1.10	96.0	1.2	4.0	1.2	<1		95.6	0.5	90.6	1.1	0.81[c]	0.05

[a]Cells lavaged from six mice, except for four mice used for total cell count after 10 exposures to PAN.
[b]Percent of AMs engulfing at least one latex sphere after 1 h incubation at 37°C.
[c]Significant change from corresponding mice exposed to air ($p < 0.05$).

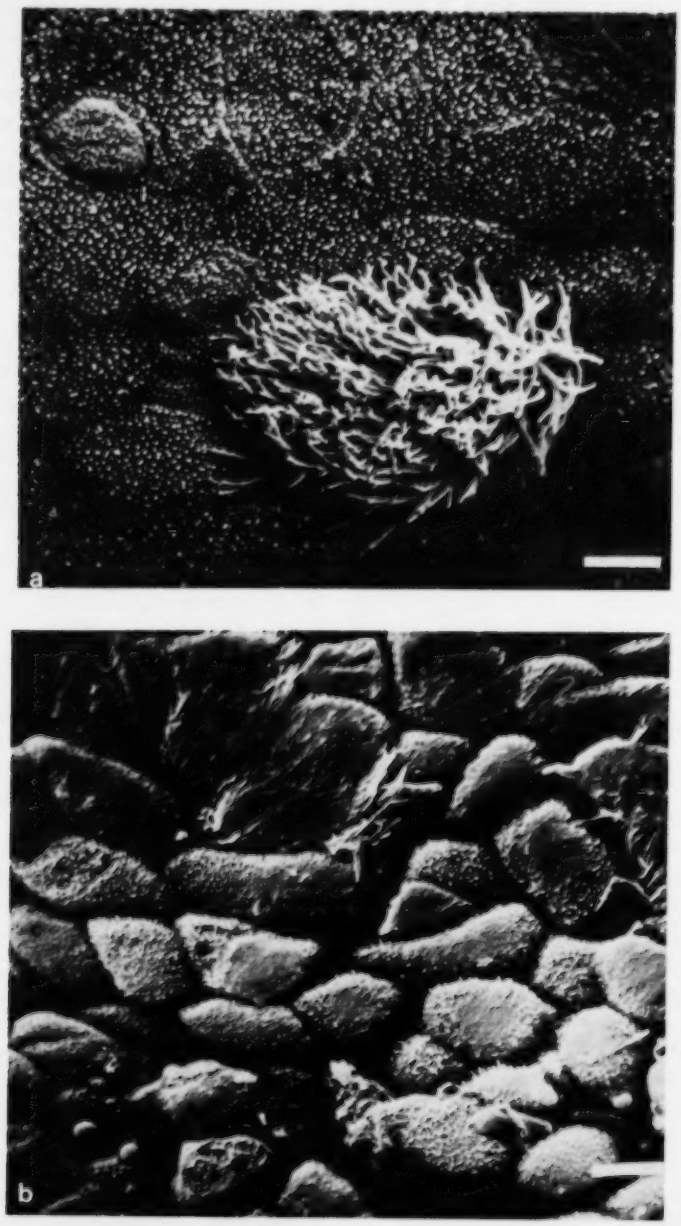

FIGURE 2. Nonciliated epithelial cells in nasal cavities of mice: (*a*) normal; (*b*) raised and clumped in mice exposed for 3 h to 25 mg/m^3 PAN. Scale bar, 5 μm.

cells with some sloughing and excess mucus was apparent in the nasal cavities of mice exposed to 4.9 mg/m^3 PAN 3 h/d, 5 d/wk, for 3 wk (Figs. 3 and 4). Exposure of 6-wk-old mice to 7.4 mg/m^3 PAN for 3 h/d, 5 d/wk, for 2 wk resulted in similar damage to the nonciliated cells of the trachea. In 16-wk-old mice under the same exposure regimen the nasal cavity tissues, tracheas, and lungs appeared normal. Thus although multiple exposures to PAN at a low concentration did not cause increased mortality due to streptococcal infection, there was evidence of morphological damage to cells of the respiratory tract and to free cells lavaged from the lungs.

DISCUSSION

A single 2- or 3-h exposure to PAN at 14.8–28.4 mg/m^3 caused a significant increase in the susceptibility of mice to streptococcal pneumonia. The mortality rate increased linearly with increasing CT index. The concentration of O_3 that produced a similar or greater percentage of excess mortality was 0.98 mg/m^3, much lower than that of PAN. Thus our results agree with those of others (National Air Pollution Control Administration, 1970) that PAN is less toxic than O_3 for mice.

Multiple exposures to lower concentrations of PAN did not increase the mortality of mice due to streptococcal pneumonia. Apparently the concentration of pollutant was more significant than the duration of exposure, since CT indices of 45–85 caused significant increases in mortality on a single exposure, whereas indices $\geq$147 at the lower concentrations had no effect. Gardner et al. (1979) observed the same phenomenon in similar studies with NO_2. Although gross changes in susceptibility to streptococcal infection were not observed with these lower PAN concentrations, more subtle alterations in free cells lavaged from the lungs and damage to tissues of the respiratory tract were evident. Multiple exposures of mice to 7.4 mg/m^3 PAN for 10 d resulted in decreased leukocytes in lung lavage fluid and significantly lower levels of ATP in the AMs. The pollutant also decreased the ability of the AMs to attach to glass. Numerous studies have shown that *in vivo* and *in vitro* exposure to O_3 or NO_2 can alter the total and differential cell counts of lung lavage fluid as well as phagocytic ability, enzyme levels, and morphology of AMs (Acton and Myrvik, 1972; Gardner et al., 1969; Henry et al., 1973; Goldstein et al., 1978; Hurst et al., 1970; Aranyi et al., 1976).

Scanning electron microscopy revealed damage to the ciliated and nonciliated epithelial cells of the nasal cavities or tracheas of 6-wk-old mice exposed to both single high and multiple low concentrations of PAN. Older mice (16 wk) did not appear to sustain damage to cells of the respiratory tract. The older animals may have been more resistant to lower concentrations of PAN, although the data on excess mortality from streptococcal infection with single exposures to PAN showed no difference in mice of various ages. These studies did not attempt to demonstrate the role of age

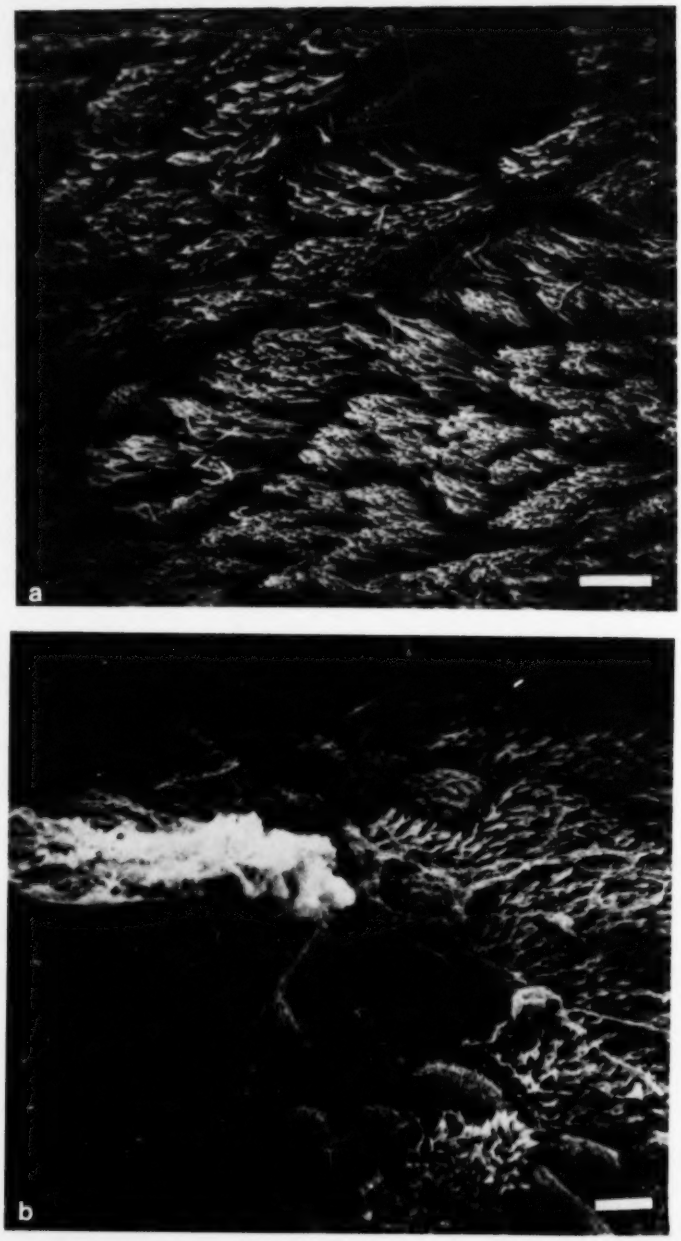

FIGURE 3. Ciliated epithelial cells in nasal cavities of mice: (a) normal; (b) excess mucus in mice exposed to 4.9 mg/m^3 PAN, 3 h/d, 5 d/wk, for 3 wk. Scale bar, 5 μm.

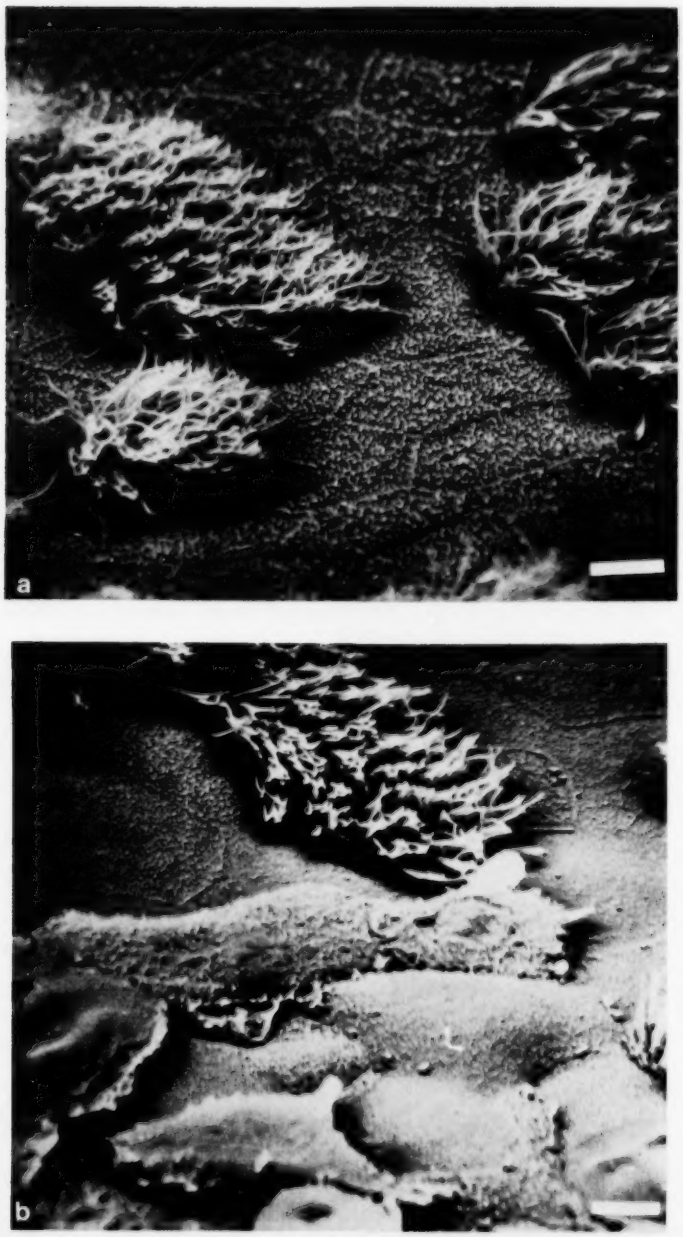

FIGURE 4. Nonciliated epithelial cells in nasal cavities of mice: (a) normal; (b) raised and sloughing in mice exposed to 4.9 mg/m^3 PAN, 3 h/d, 5 d/wk, for 3 wk. Scale bar, 5 μm.

in susceptibility of mice to PAN; for that study a wider range of ages should be employed. However, we did not find, as did Campbell et al. (1967), that older mice were more susceptible than younger mice to the toxicity of PAN.

Exposure to 25 mg/m^3 PAN for 3 h daily for 6 d did not alter the susceptibility of mice to tuberculosis, a chronic respiratory infection. Similar results were obtained when mice were exposed to 0.98 mg/m^3 O_3 for 3 h/d, 5 d/wk, for 7 wks (unpublished data). However, when the O_3 concentration was increased to 1.96 mg/m^3 for the same period, significant enhancement of *M. tuberculosis* titers in the lungs of mice was found. Limited supplies of pure PAN prevented comparable long-term exposures.

Since pure PAN is difficult to obtain and to work with, a limited number of studies have been performed by others. Previously, mice were exposed either to very high concentrations (about 700 mg/m^3) or to lower concentrations (~ 75 mg/m^3) for periods of many months to achieve toxic effects. In the experiments reported here, we employed a sensitive model of host resistance to bacterial infection to show toxic effects of various concentrations of PAN given for periods ranging from 2 h to multiple 3-h exposures for 3 wk. Our studies demonstrated subtle damage to leukocytes of the lungs and tissues of the respiratory tract as well as significantly increased mortality and decreased survival time due to exposure to PAN.

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